Supplemental Online Content

Lim YY, Maruff P, Barthélemy NR, et al; Dominantly Inherited Alzheimer Network. Association of *BDNF* Val66Met with tau hyperphosphorylation and cognition in dominantly inherited Alzheimer disease. *JAMA Neurol*. Published online January 31, 2022. doi:10.1001/jamaneurol.2021.5181

eMethods.

eTable 1. Demographic, Clinical, and Biological Characteristics of the ADNI Preclinical Sporadic AD Sample

eTable 2. Effect of *BDNF* Val66Met on Baseline Episodic Memory, and Levels of CSF Total Tau and CSF p-Tau181 in Preclinical Sporadic AD

eTable 3. Effect of Clinical Group and EYO on Cognitive Outcomes, Site-Specific Tau Phosphorylation Occupancies, and MS Total Tau Levels

eTable 4. Effect of Clinical Group and EYO on Each Cognitive and Biomarker Outcome in *PS1* Mutation Carriers

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

Each thawed CSF sample was mixed with 25 µl of a solution containing 15N-441 tau internal standard (2.5 ng per sample), 50 mM guanidine, 10% NP-40 and 10×protease inhibitor cocktail (Roche). Tau was extracted by immune capture using incubation under rotation at room temperature for 2 h with 20 µl of Sepharose beads cross-linked to Tau-1 (tau epitope 192–199) and HJ8.5 (tau epitope 27–35) antibodies. Beads were spun by centrifugation, then rinsed three times with 1 ml of 25 mM triethylammonium bicarbonate. Samples were digested overnight at 37°C with 400 ng of trypsin Gold (Promega). AQUA peptides (Life Technologies) were spiked to obtain an amount of 5 fmol per labeled phosphorylated peptide and 50 fmol per labeled unmodified peptide in each sample. The peptide mixture was loaded on TopTip C18 tips, washed with 0.1% formic acid solution and eluted with 60% acetonitrile/0.1% formic acid solution. Eluates were dried using a Speedvac and dried samples were stored at −80 °C before analysis. Samples were resuspended in 25 µl of 2% acetonitrile/0.1% formic acid. Extracts were analyzed by nano liquid chromatography coupled to high-resolution tandem mass spectrometry (HRMS/MS) using parallel reaction monitoring using HCD fragmentation. Nano liquid chromatography–HRMS/MS experiments were performed using a nanoAcquity UPLC system (Waters) coupled to a Fusion Tribrid mass spectrometer (Thermo Fisher Scientific). For each sample, 5 µl was injected. Peptide separation was achieved at 60 °C in 24 min on a Waters HSS T3 column (75 µm × 100 mm; 1.8 µm). Mobile phases were: (A) 0.1% formic acid in water; and (B) 0.1% formic acid in acetonitrile. The gradient used was 0.5% B at 0 min, 5% B at 7.5 min and 18% B at 22 min, then the column was rinsed for 2 min with 95% B. The flow rate was set at 700 nl/min for 7.5 min, then 400 nl/min for the rest of the analysis. Data were acquired in the positive ion mode at a spray voltage of 2,200 V (Nanospray Flex ion source; Thermo Fisher Scientific) and the ion transfer tube was set at 270 °C. The S-lens radio frequency voltage was set at 60 V. HRMS/MS transitions were extracted using Skyline software (MacCoss laboratory). CSF tau phosphorylation levels were calculated using measured ratios between HRMS/MS transitions of endogenous unphosphorylated peptides and 15N-labeled peptides from the protein internal standard. Ptau/tau ratios defining site phosphorylation occupancy on T181, T217, and T205 were measured using the ratio of the HRMS/MS transitions from phosphorylated peptides and the corresponding unphosphorylated peptides. Each phosphorylated/unphosphorylated peptide endogenous ratio was normalized using the ratio measured on the HRMS/MS transitions of the corresponding AQUA phosphorylated/unphosphorylated peptide internal standards.

eTable 1. Demographic, clinical and biological characteristics of the ADNI preclinical sporadic AD sample.

	CN Val66 homozygotes (n=87)	CN Met66 carriers (n=38)	
	N (%)	N (%)	*p*
Female	46 (52.9%)	19 (50.0%)	.767
APOE ε4	31 (35.6%)	20 (52.6%)	.075
	Mean (SD)	Mean (SD)	*p*
Age	74.73 (5.21)	74.98 (6.46)	.822
Education	16.24 (2.67)	16.32 (2.09)	.879
MMSE	29.13 (1.08)	29.11 (1.13)	.921
CDR Total	0.00 (0.00)	0.00 (0.00)	.999
GDS	0.76 (0.93)	0.89 (1.31)	.509
CSF $A\beta_{42}$ (pg/ml)	759.26 (199.68)	713.62 (209.24)	.249

*Note: CN = cognitively normal; APOE = apolipoprotein E; MMSE = Mini Mental State Examination; CDR = Clinical Dementia Rating; GDS = Geriatric Depression Scale; CSF = cerebrospinal fluid; $A\beta_{42}$ = $A\beta_{42}$ levels measured using immunoassay

eTable 2. Effect of *BDNF* Val66Met on baseline episodic memory, and levels of CSF total tau and CSF $ptau_{181}$ in preclinical sporadic AD (i.e., Aβ+ cognitively normal older adults).

	Episodic Memory		CSF total tau		CSF ptau181	
	(df) F	*p*	(df) F	*p*	(df) F	*p*
BDNF Group	(1,120) 3.845	.050	(1,120) 9.653	.002	(1,120) 9.893	.002
APOE ε4	(1,120) 0.905	.343	(1,120) 12.554	.001	(1,120) 13.368	.001
Sex	(1,120) 10.837	.001	(1,120) 0.840	.361	(1,120) 0.775	.380
Age	(1,120) 7.875	.006	(1,120) 10.589	.001	(1,120) 10.098	.002
	Mean (SD)		Mean (SD)		Mean (SD)	
Aβ+ Val66 homozygotes (n=87)	0.995 (0.476)		229 (93.274)		22.10 (10.260)	
Aβ+ Met66 carriers (n=38)	0.814 (0.456)		271 (90.617)		26.80 (9.925)	

*Note: Means have been adjusted for age, sex and ε4.

eTable 3. Effect of clinical group and EYO on cognitive outcomes, site-specific tau phosphorylation occupancies and MS t-tau levels

	Group		**EYO**		**Group x EYO**	
	(df) F	*p*	(df) F	*p*	(df) F	*p*
Episodic Memory	(4,306) 60.915	2.20×10^{-16}	(1,306) 14.852	1.42×10^{-4}	(4,306) 1.847	.120
Global Cognition	(4,308) 57.466	2.20×10^{-16}	(1,308) 9.512	2.22×10^{-3}	(4,308) 4.274	2.22×10^{-3}
Hippocampal Volume	(4,310) 41.601	2.20×10^{-16}	(1,310) 25.771	6.64×10^{-7}	(4,310) 5.806	1.62×10^{-4}
pT217/T217 (%)	(4,310) 127.374	2.20×10^{-16}	(1,310) 37.160	3.23×10^{-9}	(4,310) 10.981	2.43×10^{-8}
pT181/T181 (%)	(4,310) 69.576	2.20×10^{-16}	(1,310) 21.965	4.16×10^{-7}	(4,310) 7.017	2.03×10^{-8}
pT205/T205 (%)	(4,310) 104.617	2.20×10^{-16}	(1,310) 30.928	5.79×10^{-8}	(4,310) 8.527	1.54×10^{-6}
MS t-tau (T181, ng/ml)	(4,310) 41.006	2.20×10^{-16}	(1,310) 20.581	8.18×10^{-6}	(4,310) 3.858	.004
Estimated Marginal Means (SE)						
	NC	pMC Val66	pMC Met66	sMC Val66	sMC Met66	
Episodic Memory	-0.090 (0.078)	-0.036 (0.092)	-0.589 (0.126)	-1.220 (0.291)	-1.892 (0.491)	
Global Cognition	-0.350 (0.147)	-0.090 (0.162)	-0.288 (0.221)	-2.056 (0.290)	-3.383 (0.400)	
Hippocampal Volume	8.76 (0.079)	8.95 (0.093)	8.61 (0.127)	8.97 (0.295)	8.29 (0.497)	
pT217/T217 (%)	1.78 (0.215)	3.24 (0.254)	4.85 (0.346)	4.66 (0.801)	7.18 (1.350)	
pT181/T181 (%)	22.60 (0.524)	25.90 (0.619)	29.80 (0.841)	29.80 (1.952)	29.90 (3.284)	
pT205/T205 (%)	0.380 (0.019)	0.502 (0.023)	0.481 (0.031)	0.747 (0.071)	1.206 (0.120)	
MS t-tau (T181, ng/ml)	0.434 (0.022)	0.471 (0.026)	0.584 (0.035)	0.495 (0.082)	0.957 (0.138)	
Effect size of difference between selected groups of interest, Cohen's d (95% CI)						
	NC vs. pMC Val66		pMC Val66 vs. pMC Met66		sMC Val66 vs. sMC Met66	
Episodic Memory	0.06 (-0.20, 0.31)		**0.60 (0.26, 0.93)**		0.30 (-0.17, 0.77)	
Global Cognition	0.15 (-0.10, 0.41)		0.12 (-0.21, 0.45)		**0.66 (0.17, 1.13)**	
Hippocampal Volume	0.20 (-0.05, 0.46)		**0.36 (0.03, 0.69)**		0.30 (-0.17, 0.77)	
pT217/T217 (%)	**0.57 (0.31, 0.83)**		**0.63 (0.29, 0.96)**		0.41 (-0.07, 0.88)	
pT181/T181 (%)	**0.53 (0.27, 0.79)**		**0.63 (-0.29, 0.96)**		0.01 (-0.46, 0.48)	
pT205/T205 (%)	**0.53 (0.27, 0.79)**		0.09 (-0.24, 0.42)		**0.84 (0.35, 1.32)**	
MS t-tau (T181, ng/ml)	0.14 (-0.11, 0.40)		**0.43 (0.10, 0.76)**		**0.74 (0.24, 1.21)**	

*Note: bolded values indicate statistical significance at $p < .05$. Age, sex and PiB-PET SUVR were included as covariates. EYO = estimated year of symptom onset; EM = episodic memory composite; NC = non-mutation carriers; pMC = presymptomatic (CDR 0) mutation carrier; sMC = symptomatic (CDR 0.5+) mutation carrier

eTable 4. Effect of clinical group and EYO on each cognitive and biomarker outcome in PS1 mutation carriers

	Group		**EYO**		**Group x EYO**	
	(df) F	*p*	(df) F	*p*	(df) F	*p*
Episodic Memory	(4,250) 58.380	$2.20x10^{-16}$	(1,250) 12.688	$4.41x10^{-4}$	(4,250) 2.158	.074
Global Cognition	(4,253) 50.125	$2.20x10^{-16}$	(1,253) 6.803	.009	(4,253) 6.742	$3.60x10^{-5}$
Hippocampal Volume	(4,233) 40.440	$2.20x10^{-16}$	(1,233) 21.071	$7.24x10^{-6}$	(4,233) 6.031	$1.24x10^{-4}$
pT217/T217 (%)	(4,254) 115.103	$2.20x10^{-16}$	(1,254) 26.813	$4.56x10^{-7}$	(4,254) 13.156	$9.39x10^{-10}$
pT181/T181 (%)	(4,254) 68.890	$2.20x10^{-16}$	(1,254) 16.203	$7.51x10^{-5}$	(4,254) 9.295	$5.03x10^{-7}$
pT205/T205 (%)	(4,254) 107.595	$2.20x10^{-16}$	(1,254) 26.654	$4.91x10^{-7}$	(4,254) 8.680	$1.40x10^{-6}$
MS t-tau (T181, ng/ml)	(4,254) 34.922	$2.20x10^{-16}$	(1,254) 15.410	$1.11x10^{-4}$	(4,254) 3.213	.013
Estimated Marginal Means (SE)						
	NC (n=96)	pMC Val66 (n=62)	pMC Met66 (n=44)	sMC Val66 (n=38)	sMC Met66 (n=24)	
Episodic Memory	-0.015 (0.081)	-0.158 (0.115)	-0.622 (0.131)	-1.290 (0.257)	-1.655 (0.429)	
Global Cognition	-0.085 (0.203)	-0.242 (0.258)	-0.429 (0.301)	-2.503 (0.343)	-4.879 (0.425)	
Hippocampal Volume	8.86 (0.086)	8.80 (0.121)	8.43 (0.138)	8.59 (0.290)	8.47 (0.502)	
pT217/T217 (%)	1.20 (0.247)	4.44 (0.356)	5.49 (0.375)	6.73 (0.783)	7.65 (1.300)	
pT181/T181 (%)	21.30 (0.575)	28.00 (0.829)	30.80 (0.943)	33.0 (1.825)	30.30 (3.031)	
pT205/T205 (%)	0.325 (0.021)	0.537 (0.030)	0.516 (0.034)	0.792 (0.066)	1.031 (0.109)	
MS t-tau (T181, ng/ml)	0.404 (0.025)	0.529 (0.035)	0.603 (0.040)	0.611 (0.078)	0.854 (0.130)	
Effect size of difference between selected groups of interest, Cohen's d (95% CI)						
	NC vs. pMC Val66		pMC Val66 vs. pMC Met66		sMC Val66 vs. sMC Met66	
Episodic Memory	0.17 (-0.15, 0.49)		**0.52 (0.12, 0.91)**		0.20 (-0.31, 0.71)	
Global Cognition	0.08 (-0.24, 0.40)		0.09 (-0.29, 0.48)		**1.13 (0.57, 1.66)**	
Hippocampal Volume	0.07 (-0.25, 0.39)		**0.39 (0.00, 0.78)**		0.06 (-0.45, 0.57)	
pT217/T217 (%)	**1.26 (0.90, 1.60)**		**0.39 (0.00, 0.78)**		0.17 (-0.35, 0.68)	
pT181/T181 (%)	**1.12 (0.77, 1.45)**		**0.44 (0.04, 0.82)**		0.21 (-0.30, 0.72)	
pT205/T205 (%)	**0.97 (0.63, 1.30)**		0.09 (-0.30, 0.48)		**0.52 (0.00, 1.03)**	

MS t-tau (T181, ng/ml)	**0.49 (0.16, 0.81)**	0.27 (-0.12, 0.66)	0.45 (-0.08, 0.96)

* Note: bolded values indicate statistical significance at $p < .05$. EYO = estimated year of symptom onset; EM = episodic memory composite; NC = non-mutation carriers; pMC = presymptomatic (CDR 0) mutation carrier; sMC = symptomatic (CDR 0.5+) mutation carrier